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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/019,409	12/28/2001	Masahiro Iwakura	04853.0084	3873

22852 7590 04/25/2005

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EXAMINER	
PROUTY, REBECCA E	
ART UNIT	**PAPER NUMBER**
1652	

DATE MAILED: 04/25/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/019,409	IWAKURA, MASAHIRO	
	Examiner	Art Unit	
	Rebecca E. Prouty	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 February 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *11-14* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *11-14* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

RD

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 2/3/05 has been entered.

Claims 1-10 have been canceled. Newly presented claims 11-14 are at issue and are present for examination.

Applicants' arguments filed on 2/3/05, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 11-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combined disclosures of Li et al. and

Recktenwald et al., in view of Lathrop et al. and Barnett et al. (WO96/30481).

Li et al. and Recktenwald et al. teach that the oxidative lability of industrial and pharmaceutical enzymes is a well known problem in the art and that in particular the amino acid residues methionine and cysteine are highly oxidatively labile (see particularly Table 2 of Recktenwald et al. and the abstract and pages 491-493 of Li et al.). Li et al. and Recktenwald et al. further teach that the site-specific substitution of methionine and cysteine residues has been a known strategy for the improvement of the oxidative stability of a variety of proteins of interest (see Table 2 and pages 7-8 of Recktenwald et al. and the abstract and pages 496-497 of Li et al.) and that this strategy has been successfully applied to at least subtilisin (Recktenwald et al., page 8) and α_1-antitrypsin (Li et al., page 497).

Barnett et al. teach the mutagenesis of *Bacillus* α-amylases to improve oxidative stability. Barnett teach that oxidative stability can be improved by the site-specific substitution of oxidizable amino acids such as methionine, tryptophan, tyrosine, histidine and cysteine with non-oxidizable amino acids (pages 2 and 4) and that in particular cysteine and methionine are the most oxidizable amino acids (page 4). Barnett et al. further

teach the alteration of multiple amino acids within the enzyme to achieve the maximum stability (page 15). Barnett et al teach that this alteration of the enzyme can be accomplished while maintaining adequate enzymatic activity compared to the wild-type enzyme (page 3 and examples 11, 14, 16 and 17). Barnett et al. teach specific methods of constructing multiply substituted site-specific mutants which include a) constructing all possible single site mutants at several distinct methionine residues, selecting those single mutants with the highest activity and then screening combinations of these mutations for those multiple mutants with the highest activities (see particularly Examples 3, 5-7, and 11) and b) creating double mutants by further mutating a precursor single mutant enzyme (see particularly Example 10).

In view of the combined disclosures of Li et al. and Recktenwald et al. the ordinary skilled artisan would have found it obvious to use site specific mutagenesis to replace all the methionine and cysteine residues of any enzyme which is used in oxidative conditions with more oxidatively stable amino acids in order to improve the oxidative stability of the protein. Furthermore, the skilled artisan would have found it obvious to use either of the methods of making multiple mutants taught by Barnett et al. for making multiple mutants of *Bacillus* α-

amylase, as Barnett et al. showed that these methods resulted in oxidatively stable enzymes with activity at least equal to that of the wild type enzyme. As ATG (which encodes methionine) is the initiation codon of virtually all known genes, one of ordinary skill in the would have recognized that replacing all methionine residues would require a means for altering the N-terminal methionine while still allowing for translation initiation.

Lathrop et al. teach a method for mutating a recombinant protein produced in *E. coli* such that the methionine residue encoded by the initiation codon will be cleaved by the methionine aminopeptidase activity of the host cell. Lathrop et al. teach altering the penultimate amino acid residue codon (i.e., the residue immediately following the initiation codon) such that the first two amino acids encode a sequence which is a good substrate for *E. coli* methionine aminopeptidase and screening for a mutant enzyme that lacks an amino-terminal methionine residue but maintains activity.

Therefore, it would have been further obvious to one of skill in the art to combine the method of Lathrop et al. with the mutagenesis methods discussed above to construct an enzyme lacking any methionine residues. One of skill in the art would have been motivated to do so by the disclosures of Li et al. and

Recktenwald et al. that the oxidative lability of industrial and pharmaceutical enzymes is a well known problem in the art which can be overcome by replacement of oxidatively labile amino acid residues.

Applicants argue that the prior art does not teach or suggest replacing all methionine and cysteine residues in a single protein. Rather, the combined disclosures teach the selective replacement of particular methionine or cysteine residues based on rational criteria and, therefore, teach away from replacing all methionine and cysteine residues in a single protein. The examiner acknowledges that the prior art that was cited focuses on the mutagenesis of one or a few oxidatively labile amino acids in contrast to applicants methods which recite the alteration of all sulfur containing amino acids in the enzyme of interest. However, the art clearly teaches and suggests the alteration of multiple residues and particularly, all residues which contribute to oxidative lability. Furthermore, it is clear that in the art for the vast majority of proteins there is only limited structural information available (see for example page 2 of Recktenwald et al.) such that information as to which amino acids in a protein are in fact responsible for any observed oxidative lability is not available. In this situation, the skilled artisan would be

faced with two choices, trying to experimentally determine which residues contribute to the oxidatively lability or alternatively replacing them all. As site specific mutagenesis techniques have advanced such that multiple mutagenesis and screening can be done quickly and cost-effectively, the skilled artisan would be more motivated to choose the mutagenesis of all residues than the experimental determination of which residues to focus on as determining which residues contribute would be difficult unless only one residue appears to be particularly labile (as was found for the protein of Barnett et al.). Applicants claimed methods amount merely to a systematic approach to accomplishing the goal of mutating all possible oxidatively labile residues in a manner in which some activity of the enzyme can be maintained. Both applicants "combined mutation method" i.e., determining individually the most active replacements at each position individually and then combining these together and applicants "stepwise method", i.e., further mutagenizing a previously selected mutant, of modifying would have been obvious to a skilled artisan given a desire to mutate any group of several amino acids in a protein while maintaining activity and both strategies have been used in the art for multiple mutagenesis experiments. The cited art unquestionably teaches the combination of individually selected best variants at several

positions while well known mutagenesis methods such as "directed evolution" make it clear that a skilled artisan would consider multiple repetitive rounds of mutagenesis and selection as in the stepwise method as well.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Rebecca E. Prouty whose telephone number is 571-272-0937. The examiner can normally be reached on Tuesday-Friday from 8 AM to 5 PM. The examiner can also be reached on alternate Mondays

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (571) 272-0928. The fax phone number for this Group is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Rebecca Prouty
Primary Examiner
Art Unit 1652